Complex behavioral manipulation drives mismatch between host and parasite diversity

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2 **diversity**

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4 **Short title:** Mismatch between host and parasite diversity

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25   **Abstract**

26   Parasites and hosts are intimately associated such that changes in the diversity of one

27   partner are thought to lead to changes in the other. We investigated this linked

28   diversity hypothesis in a specialized ant-*Ophiocordyceps* system in three forests

29   across 750 km in Central Amazonia. All species belonging to the fungal genus

30   *Ophiocordyceps* associated with ants have evolved some degree of behavioral control

31   to increase their own transmission, but the leaf-biting behavior is the most complex

32   form of host manipulation. Such a system requires control of the mandibular muscles

33   and a distinct shift in behavior, from climbing vegetation to walking on leaves to

34   rasping leaf veins in the seconds before death. The need to induce complex behavior

35   may limit host availability and represent a constraint on parasite diversity. The

36   consequence for community structure is that complex behavioral manipulation leads

37   to a mismatch between ant hosts and the diversity of their fungal parasites.

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39   **Keywords:** ants, behavior manipulation, interaction, Ophicordyceps, tropical forests.

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42   **Introduction**

43   Species diversity varies considerably between habitats and regions [1], and the

44   factors driving such heterogeneity typically depend on the scale of the analysis [2]. At

45   the local level, interactions among species are known to play an important role in

46   structuring communities [3]. This is especially the case when the interacting species

47   occupy different trophic levels, which leads to a stronger link between the diversity of

48   consumers and the diversity of resources [4–6]. Parasite-host interactions are

49   examples of such trophic effects. Parasites tend to be host specific with hosts serving

50   as both the habitat and the dispersal agents for parasites [7]. This implies that changes

51   in host abundance often lead to changes in parasite abundance. Such specificity is

52   considered to lead to arms races that promote overall diversity at the community level

53   [8].

54   Within the framework of linked diversity in host-parasite systems, the sub-set

55   of parasites that manipulate behavior has not been considered. The effect of parasites

56   on their hosts is not only to reduce host fitness but in some cases also involves a

57   manipulation of host behavior that directly increases parasite fitness [9]. In these

58   cases, other constraints acting on the parasite related to its need to control behavior as

59   a life-history strategy may affect the coupling of diversity across scales. The

60   interaction between ants and the ascomycete fungus *Ophiocordyceps* provides a

61   convenient model for understanding the roles of behavioral manipulation on patterns

62   of host and parasite diversity. Ants infected by *Ophiocordyceps* species die in specific

63   locations outside the nest where the microenvironment is ideal for fungal sporulation

64   and subsequent dispersal to new hosts [10-12]. Dying outside the nest is considered

65   adaptive for the fungus, because it avoids the cleaning behavior of ant workers that

66   may prevent the completion of the fungal lifecycle inside the colony [10]. Depending

67   on which fungal species is involved, infected ants may die attached to stems (Fig.

68   1C), buried in the leaf-litter, attached to tree bark (Fig. 1D) or biting leafs (Fig. 1E).

69   Biting leaf veins or leaf tissue is the most complex form of manipulation and

70   maintains the ant in situ after death giving the fungus the necessary 24-48 hours to

71   grow adhesive mycelia that bind the ant to the plant [11]. This behavioral

72   manipulation is ancient with vein biting occurring at least since the Eocene [12].

73   Recent evidence has shown that this host-parasite relationship is highly specific with

74   each host species examined having its own specific parasite species [13].

75   To explore the linked diversity hypothesis between parasite and host we

76   worked with a large dataset of more than 70,000 samples representing 340 ant species

77   with knoweldge on parasite diversity built up from 2,700 samples collected from three

78   Amazonian sites across a 750km transect (Fig. 1A). We specifically compare the

79   composition of infected and non-infected ant species among sites and how the

80   complex behavioral manipulation by the fungus *Ophiocordyceps* can affect the

81   parasite assemblage structure.

82

83   **Materials and Methods**

84   We sampled ants and their fungal parasites in three Amazonian forests. Two

85   of them (Maracá Ecological Station, $3^o\,22$'N, $6^o\,127$'W and Viruá National Park, 1°

86   27'N, $61^o\,01$'W) are situated in forest reserves in Roraima State (extreme North of

87   Brazil). The third (Ducke Reserve, $2^o\,57$'S, $59^o\,56$' W) is situated 25 km North of

88   Manaus, Central Amazonia (Fig 1A). The sites cover a latitudinal gradient (~ 750 km)

89   in Amazonian forests and encompass wide environmental heterogeneity, including

90   areas of open and dense forests, and areas subject to different degrees of seasonal

91   flooding [14]. We sampled both, parasitized and non-parasitized ants in 9 plots per

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92   site covering approximately an area of 9 km^2. In each plot, we carefully searched for

93   infected ants buried in soil/litter, and attached to vegetation and tree trunks: habitats

94   where the infected ants are most commonly found. The three-dimensional volume

95   sampled per plot was ~ 500 m^3: 250 m length, 1 m wide and 2 m in height, resulting

96   in 13,500 m^3 in total. Two persons sampled each plot for at least 1.5 hours (~ 40

97   hours/person over the three sites); one focusing on all infected ants and the other on

98   non-infected ants belonging to the genera infected by *Ophiocordyceps*. In the Upper

99   Amazon, this and previous research [15] has shown that the following seven ant

100   genera are infected: *Camponotus*, *Cephalotes*, *Daceton*, *Dolichoderus*, *Ectatomma*,

101   *Pachycondyla* and *Paraponera* genera. To contrast the assemblage of infected ants

102   with the whole ant community, we used a comprehensive ant survey of 30 plots per

103   site, with over 70,000 samples collected. This survey included 900 1 m^2 litter samples

104   (Winkler sacks), 900 pitfall-traps and 900 sardine baits regularly distributed among

105   the three sites to describe the ant assemblage composition (see [14] for additional

106   details).

107   The data were organized in three matrices: 1) all ant species collected at the

108   three sites, 2) all species from the genera known to be suitable hosts (i.e. *Camponotus*,

109   *Cephalotes*, *Daceton*, *Dolichoderus*, *Ectatomma*, *Pachycondyla* and *Paraponera*

110   genera) and 3) only the species we discovered to be infected. This last category was

111   created because not all species in a genus are infected. To provide further

112   understanding of the role of complex behavioral manipulation on ant community-

113   level patterns, we also constructed matrices of ant species according to the type of

114   manipulation (Fig 1B). We reduced the dimensionality of all matrices using Principal

115   Coordinate Analysis (PCoA) based on the Sørensen dissimilarity index.

116   Presence/absence data were used to avoid overestimation of species with larger nests.

117   We compared assemblage composition between the three areas using non-parametric

118   multivariate analysis of variance [16]. The statistical significance of each analysis was

119   based on 9,999 Monte Carlo permutations.

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122   **Results**

123   We found that the ant assemblage composition was markedly different

124   between areas (npMANOVA, $p < 0.001$; Fig 1F). We recorded 343 species from 58

125   different genera in 11 sub-families. We found very little overlap of species between

126   areas; only 72 out of 343 ant species (~20%) were sampled in each of the three sites.

127   Although we recorded 58 genera of ants only 7 genera contain species that are

128   infected by *Ophiocordyceps* (these are *Camponotus*, *Cephalotes*, *Daceton*,

129   *Dolichoderus*, *Ectatomma*, *Pachycondyla* and *Paraponera*). The assemblage

130   composition of 68 species of ants belonging to these 7 genera also were different

131   between areas (npMANOVA, $p < 0.001$, Fig S1). However, the assemblage of

132   infected ants did not mirror the community structure; either of all ants from all genera

133   or all ants from the genera that we identified as containing infected species. The

134   assemblage composition of species of ants that are infected by *Ophiocordyceps* was

135   not different between sites (npMANOVA, $p = 0.109$; Fig 1G). Put another way,

136   despite the fact that ~20% of the ant species were shared among the three locations

137   the infected ant species were similar between these three very geographically and

138   ecologically different sites. We had expected that different areas would have different

139   infected species assemblages reflecting the general pattern of ant diversity across

140   three sites, i.e. linked diversity between hosts and parasites. The linked diversity in

141   the host-parasite system only matched when the infected ants that are manipulated to

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142 bite into plant tissue are removed from the analysis (npMANOVA, $p < 0.001$, Fig.

143 1H).

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145 **Discussion**

146   To infect an ant worker, *Ophiocordyceps* fungus produces spores that are

147 released onto the forest floor or onto vegetation, over both of which foraging ants

148 move. The spores of this fungus are very large, with a relatively thin cell wall and

149 devoid of pigmentation [i.e. hyaline 17], making them sensitive to dehydration and

150 UV radiation. Therefore, for fungal life cycle completion, an ant must pass, at the

151 correct time of the day, over the spores scattered on the forest floor to be infected. In

152 addition, the parasite must overcome the host population structure to complete their

153 life cycle. Ants are very interactive organisms, and the competition between colonies

154 of the same species at local scale has been frequently demonstrated [18]. Colony-

155 colony competition implies an additional barrier for the transmission between

156 colonies of a specialized parasite. However, the complex behavioral manipulation by

157 *O. unilateralis* seems to circumvent these barriers by creating a relative large

158 minefield areas (~ 30m^2, [11]) where the foraging ants can be infected by spores. This

159 is achieved by very high densities of manipulated/killed hosts in a small area known

160 as graveyards (Pontop et al).Virtually, in all the plots where we found species of ants

161 known to be a host to *O. unilateralis* complex we also found ants infected by fungi

162 within the *O. unilateralis* complex. And over the 750 km range the same group of

163 *Camponotus* species were infected despite those areas having limited overlapp in

164 *Camponotus* species asssemblage (Fig 2). Other groups of *Ophiocordyceps* that infect

165 ant species with large colonies, such as the ant genera *Cephalotes* and *Dolichoderus*

166 (both included in this study) also create graveyards, but in smaller areas (personal

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167   observations). In the latter case, the dead ants died attached in one tree trunk [17], and

168   sites without any infected ants were more common (Fig 2).

169   Biting behavior requires a control of the mandibular muscles that involves a

170   reduction in muscle organelle abundance [12]. It also requires a distinct shift in

171   behavior in the seconds before biting as infected ants shift from a wandering behavior

172   to rasping of either the major veins or leaf edges. Other complexes of *Ophiocordyceps*

173   cause ants to die on leaves (*O. lloydii*, 17) but the do not cause ants to bite into the

174   plant tissue. We suggest that the nature of complex manipulation and the necessary

175   additional control of the host's phenotype that is entailed limit the potential host range

176   of fungi investing in manipulation. Transmission requires this complex control of

177   behavior, which in turn requires multiple effects at the physiological and neuronal

178   level. The consequence for community structure is that even across large geographical

179   areas, complex behavioral manipulation results in a mismatch between host and

180   parasite diversity patterns. However, despite this apparent constraint the evolution of

181   behavioral manipulation seems to be a successful strategy and ant hosts of *O.*

182   *unilateralis* group were by far the most abundant hosts we discovered.

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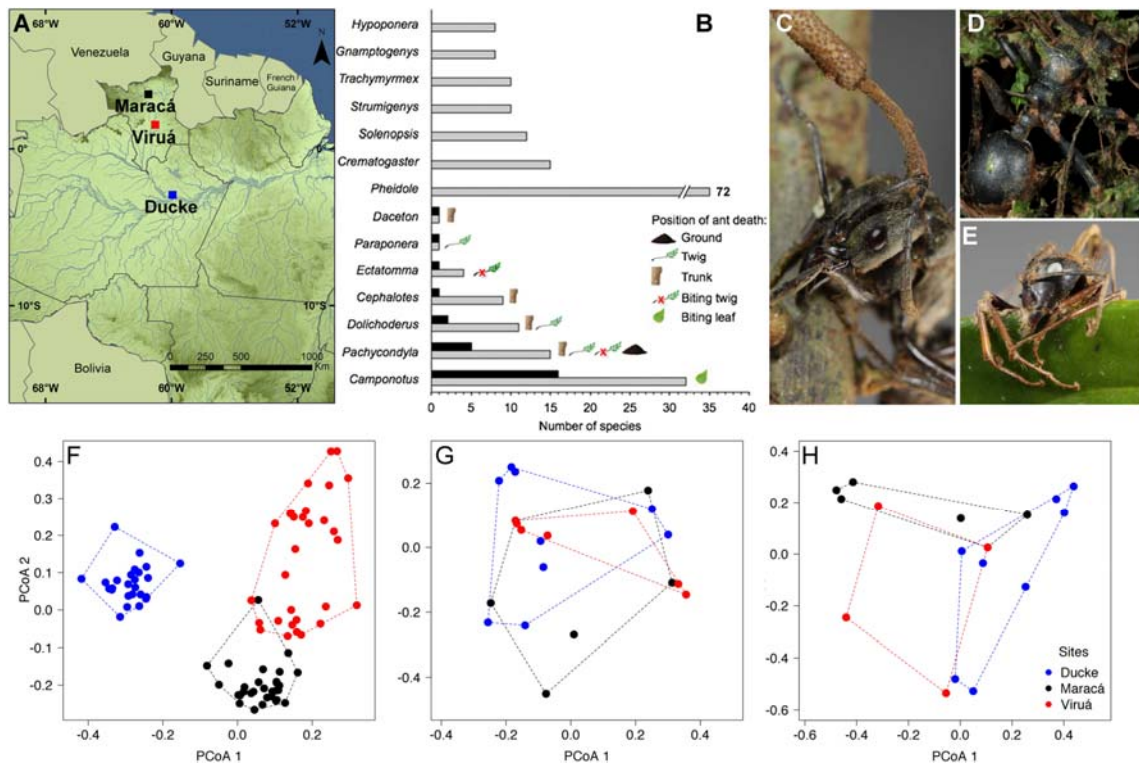
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Figure 1. (A) Map of study area. (B) Relative infection levels by ant genera showing where the ants died and the seven more specious non-infected genera sampled in 27 plots (note that *Pheidole* bar is at different scale). Black bar shows the number of infected species and gray bars the number of non-infected species. (C) *Pachycondyla inversa* infected by *Ophiocordyceps kniphofioides* var. *ponerinarum* attached to a stem. (D) *Cephalotes atratus* killed by *O. kniphofioides* var. *kniphofioides* buried in the mosses of a tree trunk. (E) *Camponotus atriceps* parasitized by *O. unilateralis s.l.* biting a leaf edge. PCoA ordination plots indicating (F) the differences in species composition among the three sites using all data, (G) congruence in species composition of all infected ant species found in 25 out of 27 plots and (H) different assemblage composition of non-biting infected ants. Some plots are stacked in the last two figures, because had the same infected ant species composition.
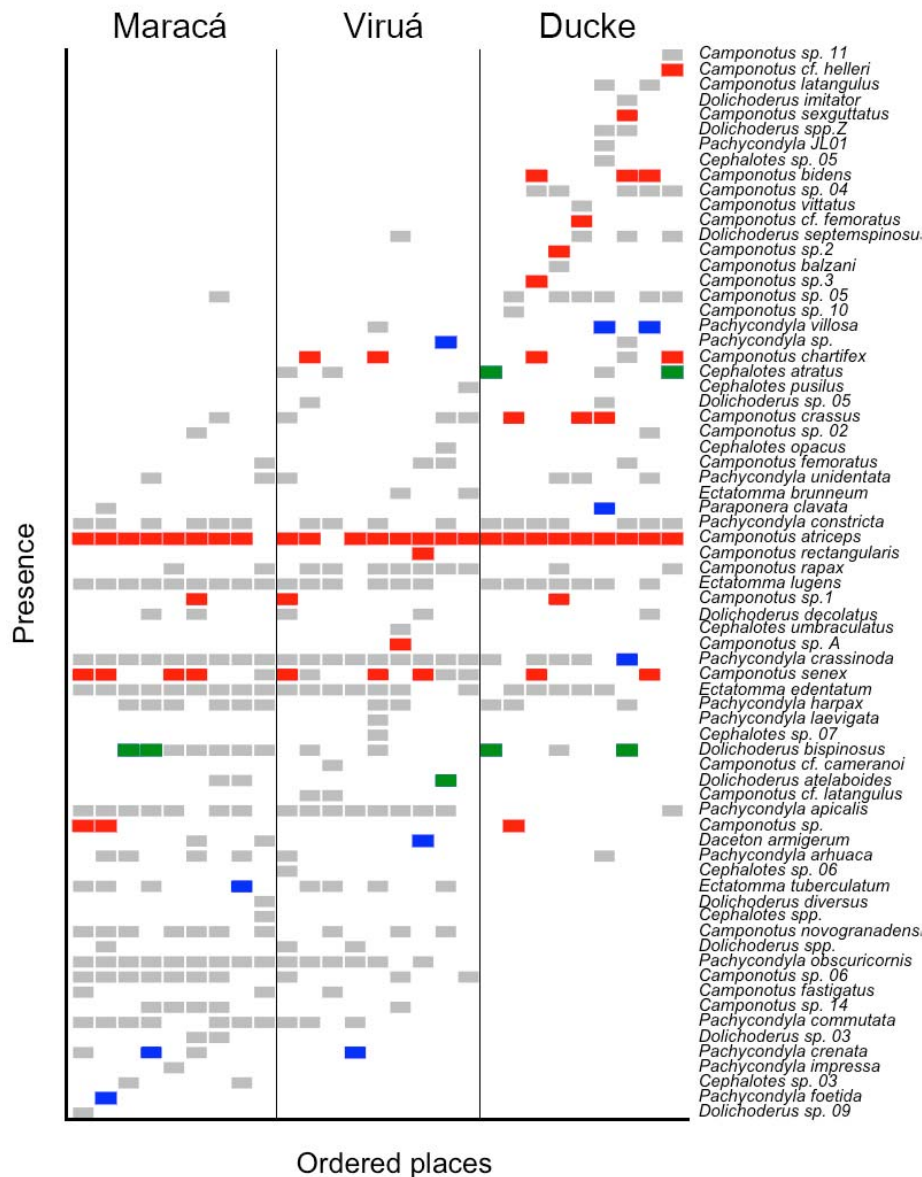
Figure 2. Distribution of ant species ordered by the first axis of PCoA analysis in study plots at Maracá, Viruá and Ducke sites. Occurrence of uninfected ants are in gray. Plots where ant species were infected by *unilateralis* complex (biting plant tissue) are showed in red. Blue and green bars represent plots were ants infected by *australis* and *kniphofioides* complex were found, respectivetly. In the later case, the infected ants were found on litter and buried in tree trunks.